

A High Serum Level of Taurocholic Acid is Correlated with the Severity and Resolution of Drug-induced Liver Injury

Short title: Taurocholic acid and the severity of DILI

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29 **Abbreviations:**

30 **ABCB11**, ATP Binding Cassette Subfamily B Member 11; **ALF**, acute liver failure;
31 **BA**, bile acid; **BSEP**, bile salt export pump; **CHB**, chronic hepatitis B; **DILI**,
32 drug-induced liver injury; **TCA**, taurocholic acid; **ULN**, upper limits of normal.

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Abstract

Background & Aims: Alterations in the serum levels of bile acids are associated with drug-induced liver injury (DILI). We investigated the association between serum levels of bile acids and the severity and outcome of DILI, along with the potential role of variants in the ATP binding cassette subfamily B member 11 (*ABCB11*) gene and expression of its product, ABCB11 (also called BSEP).

Methods: We performed this prospective study of 95 patients (median age, 53 years; 73.7% female) with DILI from August 2018 through August 2019. Patients were matched for age, gender, and body mass index with healthy individuals (n=100; healthy controls) and patients with chronic hepatitis B (n=105; CHB controls). We collected demographic and biochemical data at baseline and 1 week, 1 month, 3 months, and 6 months after DILI onset and at the time of biochemical recovery, liver failure or liver transplantation. Serum levels of bile acids were measured using high-performance liquid-chromatography tandem mass-spectrometry. All 27 exons of *ABCB11* were sequenced and expression of BSEP were analyzed by immunohistochemistry in liver biopsy specimens.

Results: Levels of 30 of the 37 bile acids analyzed differed significantly between patients with DILI and healthy controls. Changes in levels of taurocholic acid (TCA), glycocholic acid, taurochenodeoxycholate, and glycochenodeoxycholate associated with the increased levels of bilirubin and greater severity of DILI, and were also associated with CHB. Cox regression analysis showed that only change in the levels of TCA independently associated with biochemical resolution of DILI. Combination of TCA level (≥ 1955.41 nmol/L), patient age, and DILI severity was associated with abnormal blood biochemistry at 6 months after DILI onset (area under the curve, 0.81; 95% confidence interval, 0.71–0.88; sensitivity, 0.69; specificity, 0.81). *ABCB11*

84 missense variants were not associated with differences in the serum bile acid profiles,
85 DILI severity, or clinical resolution. However, lower levels of BSEP in bile canaliculi
86 in liver biopsies were associated with altered serum levels of bile acids.

87 **Conclusions:** In this prospective study performed in Chinese patients, we found that
88 the serum levels of TCA were associated with the severity and clinical resolution of
89 DILI. Reduced protein expression of BSEP in liver tissue, rather than variants of the
90 *ABCB11* gene were associated with altered serum levels of bile acids.

91 **Key words:** toxicity; herbal supplement; bile salt export pump; hepatitis B virus

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Introduction

Drug-induced liver injury (DILI) is one of the most common causes of clinically significant liver injury, with an annual incidence of 14-24 per 100,000 person-years worldwide^[1-3]. DILI can have different phenotypes with diverse clinical features and variable outcomes. Cholestatic DILI is more prone to chronicity^[4-6], whereas hepatocellular DILI has greater severity^[7] and higher mortality once jaundice develops^[8-11]. Although 87% of DILI patients achieve biochemical resolution within 6 months after withdrawal of the offending drug(s)^[3], 8% still have biochemical abnormalities within 12 months^[12], leading to chronicity or even cirrhosis. Histological characteristics could aid in the prediction of clinical outcomes^[4-6, 13, 14]. However, other noninvasive serum biomarkers are urgently needed in routine clinical practice.

Recent evidence has shown that bile acid (BA) dysregulation plays a pivotal role in DILI, as bile excretion is the major route to eliminate bilirubin and some potentially harmful exogenous lipophilic substances^[15, 16]. Animal studies have highlighted the diagnostic, prognostic and therapeutic importance of BAs in DILI^[17-22]. In a human study, serum glycodeoxycholic acid level showed prognostic value in acetaminophen-induced acute liver failure (ALF)^[23]. More recently, Ma et al. found that the serum BA levels could serve as potential biomarkers for the diagnosis and the distinction of mild from severe DILI^[24].

However, the association of altered BA profiles with the severity and prognosis of DILI warrants further verification. In addition, the potential mechanism of this

probable association remains unknown, although previous studies have illustrated the essential role of the bile salt export pump (BSEP), encoded by the ATP binding cassette subfamily B member 11 (*ABCB11*) gene, in DILI^[25, 26].

In this prospective study, we investigated the differences in BA profiles in DILI patients, healthy controls, and chronic hepatitis B (CHB) patients. Then, we explored the association of altered BA profiles with clinical phenotypes and the severity and resolution of DILI. Finally, we explored the potential roles of *ABCB11* gene variants and the liver expression of ABCB11 (BSEP) in the pathogenesis of altered serum BA profiles.

Materials and Methods

Study population

In this single-center cohort study, we prospectively recruited patients with a clinical diagnosis of DILI at Beijing Friendship Hospital, Capital Medical University, from August 2018 to August 2019.

The inclusion criteria were as follows: (1) Chinese Han nationality aged 18 to 78 years; (2) outpatients or inpatients with a diagnosis of DILI; and (3) available blood samples from the acute phase (within 10 days of obtaining the peak value of transaminase or bilirubin).

The exclusion criteria were as follows: (1) other underlying liver disease or systemic diseases affecting the liver; (2) treatment with ursodeoxycholic acid, steroids, antibiotics or probiotics two weeks prior to the collection of blood samples; (3) a steroid-dependent state; (4) cirrhosis; and (5) loss to follow-up before the study endpoints.

The diagnosis of DILI was re-ascertained^[9] by three hepatologists (QT, RY, and LW) using Roussel Uclaf Causality Assessment Method (RUCAM) score ≥ 3 (“possible”, “probable” or “highly probable” DILI). Based on the R value [ratio of alanine aminotransferase and alkaline phosphatase with their upper limits of normal (ULN)] at disease onset, DILI patients were categorized as having hepatocellular ($R \geq 5$), cholestatic ($R \leq 2$), or mixed ($2 < R < 5$) biochemical phenotypes^[8]. The disease severity was evaluated according to previously reported criteria^[9].

Follow-up information was collected at 1 week, 1 month, 3 months, and 6 months

after onset until biochemical recovery^[6]. The clinical outcomes in this study included biochemical recovery, liver failure or liver transplantation.

Age-, gender- and body mass index-matched healthy individuals and a CHB cohort at the same hospital were enrolled as controls (**Supplementary Material 1**).

This study was approved by the Institutional Ethical Review Board of the Beijing Friendship Hospital, Capital Medical University [2018-P2-116-02]. All participants gave informed consent.

Measurements of serum BAs

Fasting serum samples from DILI patients during the acute phase and samples obtained from healthy controls and CHB patients were collected and frozen at -80°C for the subsequent BA measurement. Comprehensive quantitation of serum BAs was performed with high-performance liquid chromatography tandem mass spectrometry^[27] at Metabo-Profile Inc. (Shanghai, China), which was blinded to any clinical information (**Supplementary Material 2**).

Sanger sequencing of all 27 coding exons of the *ABCB11* gene

Peripheral blood mononuclear cells from the enrolled DILI patients and healthy controls were collected. Forward and reverse Sanger sequencing of all 27 coding exons of the *ABCB11* gene and the associated boundary regions (adjacent sequences in introns) were conducted (**Supplementary Material 3 & Supplementary Table 1**).

Immunohistochemical evaluation of BSEP

Percutaneous liver biopsies of DILI patients were fixed in formalin, embedded in paraffin, and sectioned at a thickness of 4 µm. BSEP expression was evaluated with

immunohistochemistry with antibody obtained from Santa Cruz Biotechnology (Shanghai) Co., Ltd., China (**Supplementary Material 4**).

Semiquantitative evaluation of BSEP staining was conducted by two liver pathologists (XZ and JL) microscopically. Normal expression was defined as no loss or a mild loss ($\leq 1/3$) of BSEP expression, and reduced expression was defined as an obvious loss ($> 1/3$) of BSEP expression. Quantitative evaluation of BSEP expression with Image-Pro Plus 6.0 (Media Cybernetics, Inc.) was conducted by a hepatologist (QT) with pathology training who was blinded to the patient data.

Statistical analysis

Categorical variables were expressed as counts or percentages, and continuous variables were expressed as the means \pm standard deviations or medians and interquartile ranges. Comparisons of different groups were performed using the chi-square test, analysis of variance or the Kruskal-Wallis test; a Cox regression model was used to identify potential risk factors associated with clinical resolution, and *Kappa* statistics were used to assess the agreement. These analyses were conducted with SPSS 24.0 (SPSS Inc., Chicago, IL).

The bioinformatic analysis was conducted using R 3.6.1 (R Foundation for Statistical Computing Platform).

The difference in diagnostic performance was assessed using receiver operating characteristic (ROC) curves; the area under the ROC (AUROC) values were compared with the Delong method using MedCalc 12.2.1.0 (MedCalc, Mariakerke, Belgium).

192 For all analyses, $P < 0.05$ was considered statistically significant.

Results

Clinical characteristics of DILI patients, healthy controls and CHB patients

This study included 95 DILI patients, 100 healthy controls (**Fig. 1**) and 105 noncirrhotic CHB patients. Age, gender, and BMI were comparable among three groups. The demographic, biochemical and clinical characteristics are summarized in **Table 1 and Supplementary Table 2-3.**

The median age of DILI patients was 53 years, and the majority (73.7%) were females. Based on the R value, 63, 17 and 15 cases were defined as having a hepatocellular, cholestatic and mixed phenotype, respectively. According to the severity evaluation, all patients were categorized as 38 mild (Grade 1), 13 moderate (Grade 2), 40 severe (Grade 3), 3 liver failure (Grade 4) and 1 fatal (Grade 5).

The median RUCAM score was 6. The majority (74.7%) of DILI cases were attributed to the use of herbs (**Supplementary Material 5 and Supplementary Table 4**). The median biochemical recovery time was 88 days; 79 cases resolved at 6 months, and 86 cases resolved at 12 months.

The BA profiles in DILI patients were significantly different from those in healthy controls

As illustrated by the principal component analysis (PCA) (**Supplementary Fig. 1A**), distinct clustering with minimal overlap was observed between DILI patients and healthy controls. Of the 37 measured BAs, the levels of 30 BAs were significantly different between DILI patients and healthy controls (**Supplementary Table 5**). The top six most significantly changed BAs with the strongest predictive ability (AUROC >

0.96) for distinguishing DILI patients from healthy controls are depicted in **Supplementary Fig. 1B & C**.

BA profiles were comparable among DILI patients with different biochemical phenotypes and with different insulting agents

DILI patients with hepatocellular, cholestatic and mixed phenotypes were comparable in terms of age, gender, and BMI (**Table 1**). The serum BA profiles could not be distinguished among the three phenotypes based on the PCA plot (**Fig. 2A**). Further analysis of the level of each BA showed that they were generally comparable across the three phenotypes.

Details of insulting agents are listed in **Supplementary Table 4**. We divided the DILI patients into three groups according to the types of insulting agents: herbs, conventional medicine and a combination of herbs and conventional medicine. As the PCA plot showed, the serum BA profiles in these groups largely overlapped (**Supplementary Fig. 2**). Further analysis also showed similar BA levels among these groups.

BA levels increased with the serum bilirubin level and could be used to distinguish the severity of DILI

The levels of five BAs, namely, taurocholic acid (TCA), glycocholic acid (GCA), taurochenodeoxycholate (TCDCA), glycochenodeoxycholate (GCDCA) and glyoursodeoxycholic acid (GUDCA), showed an increasing trend along with the serum bilirubin levels (total bilirubin <5 ULN, 5-10 ULN, 10-20 ULN and >20 ULN) (**Fig. 2B**).

Further analysis of the BA profiles in the mild (N=38), moderate (N=13), and severe/liver failure/fatal (N=44) groups of DILI patients revealed that a subgroup of BAs could distinguish different groups, as shown in the Venn diagram (**Fig. 2C**), and the details regarding the BAs are listed in **Supplementary Table 6**. Specifically, TCA, GCA, TCDCA and GCDCA could credibly determine the degree of DILI severity, as they proportionately rose with an increase in severity (**Fig. 2D**).

Notably, four patients with dismal prognoses had strikingly high TCA levels: 10152.46 nmol/L (ALF; received artificial liver support), 13742.13 nmol/L (ALF; recovered 66 days after drug withdrawal), 28537.60 nmol/L (ALF; received artificial liver support) and 26136.74 nmol/L (received liver transplantation), further corroborating the prognostic implications of serum TCA in DILI patients, as the median value of TCA in the whole DILI cohort was 1955.41 nmol/L.

Serum TCA was an independent factor that predicted the timing of disease resolution and could improve the prediction of the 6-month outcome in DILI patients

We assessed the role of clinical features (age, gender, BMI, and severity) and BA profiles in predicting the clinical course (days to biochemical normalization) with proportional Cox regression. TCA was found to be the only independent factor associated with biochemical resolution (**Table 2**).

We then analyzed the predictive ability of age, severity, TCA, and their combination for assessing the 6-month outcome (abnormal biochemistry). The AUROC values for age, severity, TCA, and their combination were 0.58, 0.65, 0.69

and 0.81, respectively (**Fig. 2E; Table 3**).

High serum TCA level alone could be a potential useful indicator for predicting 6-month abnormal biochemistry outcome with an optimal cut-off value of 1955.41 nmol/L ($\sim 2 \mu\text{mol/L}$) (AUROC=0.69, sensitivity=0.81, specificity=0.57). Furthermore, the combination of TCA, severity and age had a better predictive ability for the 6-month outcome, with an AUROC value of 0.81 and a sensitivity and specificity of 0.69 and 0.81, respectively (**Table 3**).

Changes in serum BAs along with serum bilirubin were also observed in CHB patients

The levels of several BAs, including TCA, were significantly different among healthy controls, CHB patients and DILI patients. They showed a stepwise significant increase in CHB and DILI patients compared with those in healthy controls (**Supplementary Table 7**).

Totally, the levels of seven BAs were increased along with serum bilirubin in CHB patients (**Supplementary Table 8**). Four out of these seven BAs, i.e., TCA, GCA, TCDCA, and GCDCA, were also increased along with serum bilirubin in DILI patients (**Supplementary Fig. 3**).

Although the TCA level was significantly lower in CHB patients than that in DILI patients, its increase along with serum bilirubin was similar to that observed in DILI patients. In addition, three CHB patients with acute on chronic liver failure had extremely high serum TCA levels of 15101.88 nmol/L, 11734.34 nmol/L and 7621.98 nmol/L, while the median value of TCA level in the CHB cohort was 80.14 nmol/L.

These results suggested that TCA was increased along with serum bilirubin and may be associated with liver failure without DILI specificity.

Genetic variants of *ABCB11* did not account for serum BA alterations or the severity or clinical resolution of DILI

The genetic variants in *ABCB11* gene are listed in **Supplementary Table 9**. The frequencies of all *ABCB11* missense variants in DILI patients were not significantly different from those in healthy Chinese controls. A total of 71 DILI patients (74.7%) had missense variants of *ABCB11*; however, they had comparable serum BA profiles, severity scores, and time to resolution with the remaining 24 DILI cases not having any *ABCB11* missense variant (**Fig. 3A & B**).

Reduced liver expression of BSEP was associated with serum BA alterations and disease severity

Liver biopsies were performed in 34 of the 95 DILI patients. The biopsied patients had comparable baseline characteristics with the remaining patients who declined biopsy (**Supplementary Table 10**). Hence, the biopsied patients were considered representative of the entire DILI cohort. Based on semiquantitative assessment by the liver pathologists, BSEP expression was ranked as either normal or reduced (**Fig. 3C & D**). The semiquantification of BSEP expression was validated by quantification analysis (indexed as the mean integrated optical density using Image-Pro Plus), which resulted in good interobserver agreement (*kappa* value 0.90).

The 11 DILI cases with reduced BSEP expression had significantly higher BA levels (including TCA, GCA and GCDCA) compared with the remaining 23 cases

303 with normal BSEP expression (**Fig. 3E**). In addition, severe DILI was observed in 64%
304 versus 26% of DILI cases with reduced and normal BSEP expression, respectively
305 (one-sided $P=0.042$) (**Fig. 3F**).

Discussion

In this study, we found that the levels of certain conjugated BAs increased in a stepwise manner along with DILI severity. Furthermore, the level of serum TCA was independently associated with the clinical resolution of DILI. The alteration of serum BAs was associated with reduced liver expression of BSEP but not with genetic variants of *ABCB11*, encoding BSEP. Taken together, these results suggest that a high serum TCA level is positively associated with disease severity and can potentially predict the clinical resolution of DILI patients.

In our study, TCA, GCA, TCDCA and GCDCA were found to increase along with disease severity. This is in line with a previous study that showed similar disruptions of BA levels, serving as biomarkers for the differentiation of severe and mild DILI^[24]. Similarly, Woolbright et al. found that serum glycodeoxycholic acid could predict the survival of acetaminophen-induced ALF patients^[23]. All these evidences shed light on the role of BAs in identifying high-risk DILI. Our study suggested that routinely serum test for these BAs would aid in the identification of DILI patients with greater severity or at higher risk of liver failure so that additional supportive/preventive measures could be initiated.

Moreover, our study revealed that the TCA level, as tested during the acute phase, was an independent risk factor for biochemical resolution in DILI. The TCA level alone had a similar predictive performance for the 6-month abnormal biochemistry outcome as the current constellation of severity assessment. Since the DILI severity discrimination is based on a group of parameters, including: symptoms, serum

bilirubin, blood clotting tests, hospitalization and organ dysfunction^[9, 10, 28-30], measurement of the serum TCA level may serve as a new biomarker for DILI stratification and outcome prediction. In fact, when TCA measurement was added to the current outcome assessment, the predictive performance for DILI resolution improved from 0.75 for severity-age to 0.81^[31] for TCA-severity-age, although the difference was not statistically significant. It is worth noting that the increase in serum TCA, GCA, TCDCA and GCDCA along with serum bilirubin in both DILI and CHB patients might suggest that the alteration of these BAs was not disease-specific.

In addition, we found that missense variants of *ABCB11* were not associated with serum BA alterations, disease severity or days to resolution in DILI. This finding was also consistent with those of large-cohort genome-wide association studies, which did not find genetic susceptibility of *ABCB11* in multiple-drug-induced liver injury^[32, 33]. This may serve as a strong evidence that DILI is indeed an acquired instead of inborn disease^[34]. Nevertheless, we did show that the alteration of BA profiles was partially due to the reduced expression of BSEP, a protein encoded by the *ABCB11* gene and the molecule responsible for pumping bile salts from the hepatocellular cytoplasm into the bile canaliculi^[16]. Our findings suggest that reduced protein expression of BSEP in DILI patients may occur during transcriptional and/or translational processes and/or due to the interactions between the host and the drug^[35, 36], which warrants further in-depth investigation.

The strength of this study is that it offers clinical evidence that routine measurement of certain serum BAs may improve the identification of high-risk DILI

patients and the prediction of clinical outcomes. Furthermore, we revealed that the underlying mechanism of the altered BA profiles was not due to *ABCB11* genetic variants but instead to reduced BSEP expression at the protein level.

However, the conclusion from this single-center prospective study of Chinese Han individuals with a moderate sample size needs further validation in multicenter studies. Furthermore, only 36% of the entire DILI cohort had a liver biopsy available for BSEP analysis, which may introduce possible selection bias. However, there was no significant difference in the demographic and major clinical profiles between those with and without liver biopsies; thus, we hope the BSEP immunohistochemical staining results to be interpreted as representative of the entire DILI cohort.

In conclusion, the serum levels of the conjugated BAs TCA, GCA, TCDCA and GCDCA showed a strong association with the severity of DILI. Specifically, TCA is an important biomarker that is associated with clinical resolution and can improve the prediction of the 6-month biochemical resolution outcome. The alteration of BA profiles is not due to *ABCB11* genetic variants but is due to reduced BSEP protein expression. The present findings provide further evidence that determination of the BA profile may help to optimize the management of DILI in clinical practice.

Figure Legends:

Fig. 1 Enrollment of DILI patients and healthy control subjects.¹

Fig. 2 The exploration of BAs in association with severity and days to resolution.

Overall, the levels of serum BAs in DILI patients with different biochemical phenotypes could not be distinguished as separate clusters via principal component analysis (**A**); TCA, GCA, TCDCA, GCDCA and GUDCA levels showed gradually increasing trends along with serum bilirubin levels (**B**); a subgroup of BAs could distinguish different severities of DILI (**C**); TCA, GCA, TCDCA and GCDCA rose in proportion with the increases of severity (**D**); the diagnostic performance of different indexes for predicting the 6-month abnormal biochemistry (**E**).²

Fig. 3 Role of *ABCB11*/BSEP in BA profiles, severity, and days to resolution in

DILI patients. Overall BA levels were comparable in DILI patients with or without *ABCB11* missense variants (**A**); DILI patients with or without *ABCB11* missense variants had comparable severities and days to resolution (**B**); examples of normal (**C**) and reduced (**D**) BSEP expression (immunohistochemical staining, 200×); DILI patients with reduced BSEP expression showed greater alteration of BA profiles compared with those with normal BSEP expression(**E**) and a higher risk of having a severe case (**F**); however, they had a similar number of days to resolution. No significant difference in BSEP expression was found between patients with and

¹ **HBV**, hepatitis B virus; **NAFLD**, nonalcoholic fatty liver disease; **PBC**, primary biliary cholangitis, **SSC**, secondary sclerosing cholangitis.

² **GCA**, glycocholic acid; **GCDCA**, glycochenodeoxycholate; **GUDCA**, glyoursodeoxycholic acid; **PC**, principal component; **TBil**, total bilirubin; **TCA**, taurocholic acid; **TCDCA**, taurochenodeoxycholate; **ULN**, upper limit of normal.

387 without *ABCB11* missense variants.³

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³ **GCA**, glycocholic acid; **GCDCA**, glycochenodeoxycholate; **GHCA**, glycohyocholate; **Missense (-)**, DILI patients without any *ABCB11* missense variant(s); **Missense (+)**, DILI patients with *ABCB11* missense variant(s); **Normal BSEP**, DILI patients with normal BSEP expression; **Reduced BSEP**, DILI patients with reduced BSEP expression; **TCA**, taurocholic acid.

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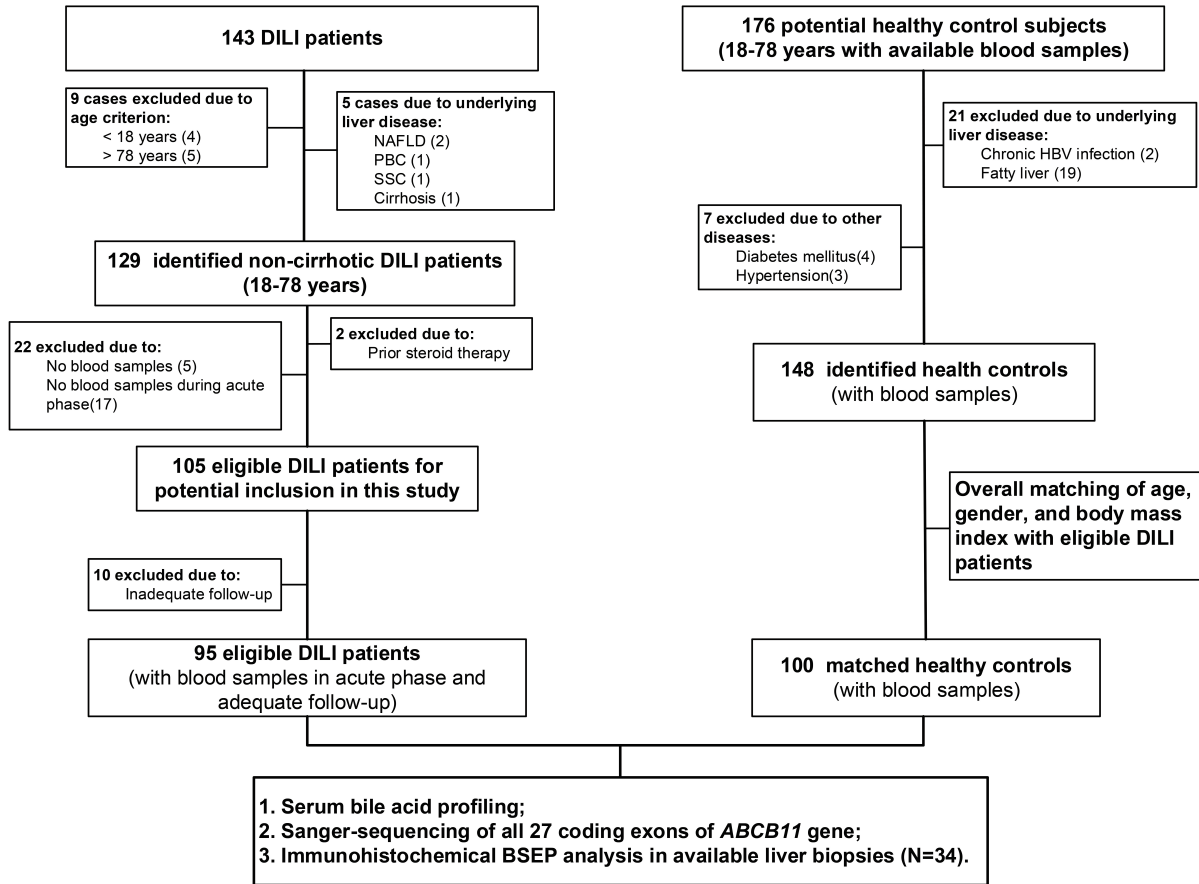
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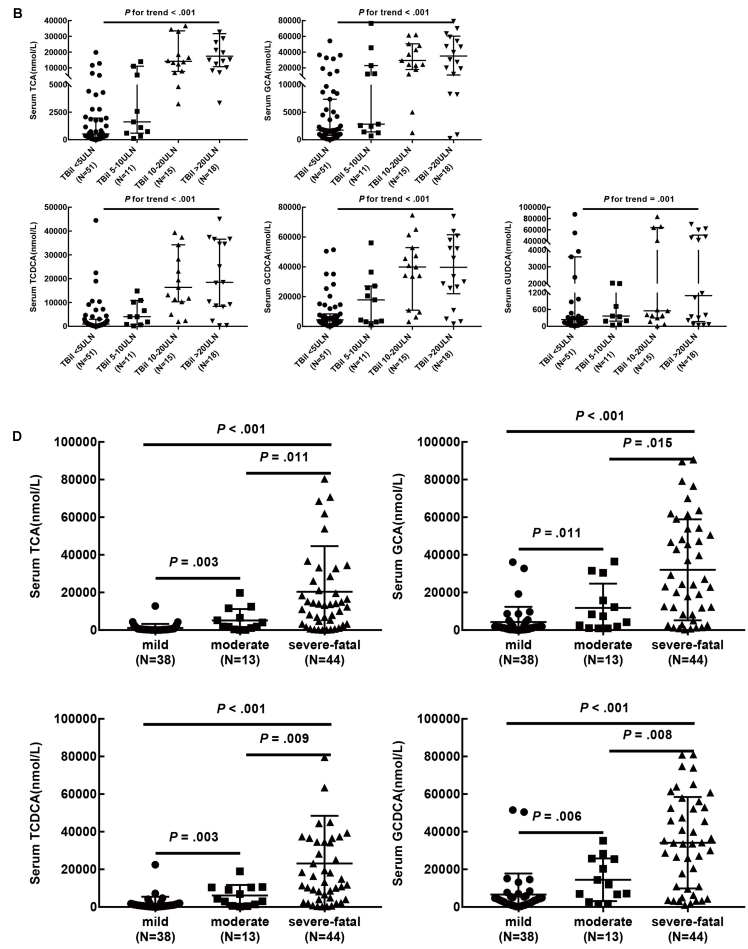
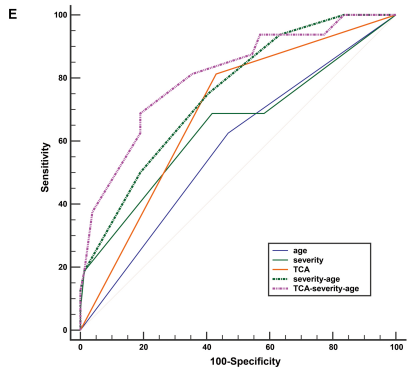
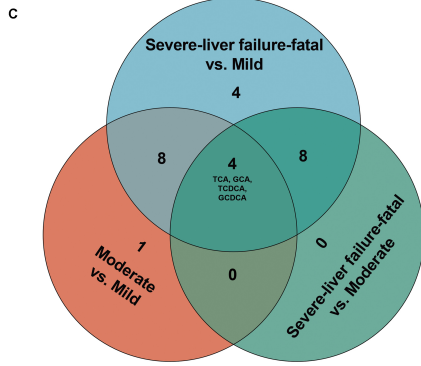
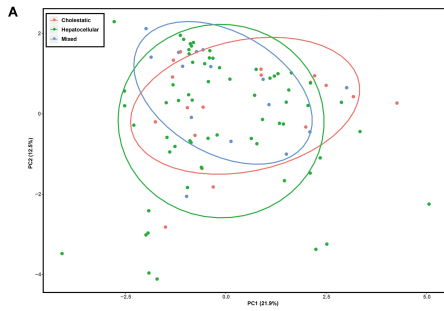
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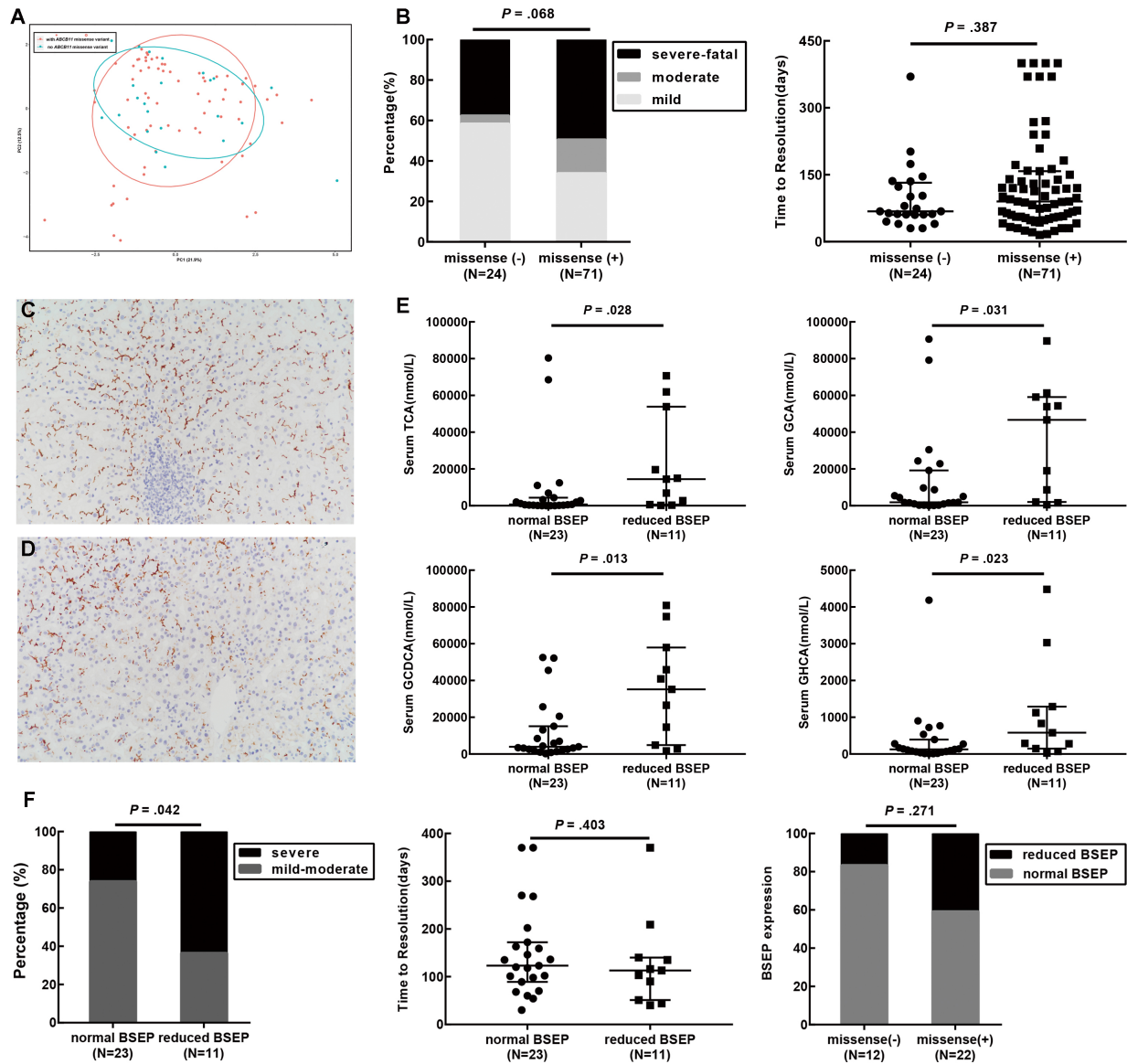


Table 1 Demographics and baseline characteristics of healthy controls and DILI patients with different biochemical phenotypes

Indexes	Healthy controls (N=100)	Overall DILI (N=95)	Hepatocellular (R≥5) (N=63)	Mixed (2<R<5) (N=15)	Cholestatic (R≤2) (N=17)	P value [#]
Age (years)	47 (45, 55)	53 (40, 63)	50 (38, 61)	63 (43, 66)	51 (48, 62)	.13
Female, N (%)	67 (67.0)	70 (73.7)	47 (74.6)	9 (60.0)	14 (82.4)	.34
BMI (kg/m ²)	23.19 (21.63, 25.50)	22.75 (20.08, 25.00)	22.80 (20.15, 25.10)	23.20 (21.03, 25.03)	21.70 (19.50, 24.00)	.45
ALT (IU/L)	27 (20, 33)	492 (172, 1026)	808 (475, 1294)	192 (111, 349)	58 (35, 79)	< .001
AST (IU/L)	28 (21, 33)	311 (110, 709)	479 (264, 1020)	132 (109, 278)	57 (43, 79)	< .001
ALP (IU/L)	91 (67, 117)	201 (126, 262)	175 (112, 223)	277 (124, 387)	246 (198, 406)	.004
GGT (IU/L)	35 (29, 43)	178 (96, 298)	169 (89, 261)	187 (87, 554)	215 (106, 307)	.46
TBil (μmol/L)	13 (10, 16)	60 (21, 187)	54 (21, 146)	165 (45, 245)	61 (19, 227)	.23
DBil (μmol/L)	8 (6, 10)	37 (7, 130)	32 (7, 93)	113 (17, 146)	31 (6, 125)	.32
TBA (μmol/L)	2.57 (2.00, 4.16)	31.85 (11.19, 151.06)	26.65 (9.80, 148.68)	29.57 (10.63, 112.70)	120.81 (15.89, 248.57)	.10
Albumin (g/L)	NT	38.4±5.0	38.9±5.1	37.5±4.2	37.4±5.3	.56
Globulin (g/L)	NT	30.5±6.8	32.1±7.3	30.9±8.3	28.5±5.7	.40
IgG (mg/dl)	NT	1312.9±343.1	1369.5±307.8	1309.5±580.5	1221.9±379.4	.65
IgM (mg/dl)	NT	98.10 (70.68, 139.40)	93.55 (66.08, 140.00)	101.00 (75.40, 130.00)	108.00 (79.30, 146.00)	.37
INR	NT	1.11 (1.01, 1.23)	1.12 (1.03, 1.23)	1.14 (0.89, 1.27)	1.04 (0.94, 1.30)	.64
ANA	NT					NA
Negative		64 (67.4)	46 (73.0)	10 (66.7)	8 (47.1)	
1:80 positive		20 (21.1)	11 (17.5)	2 (13.3)	7 (41.2)	
1:160 positive		10 (10.5)	5 (7.9)	3 (20.0)	2 (11.8)	
1:320 positive		1 (1.1)	1 (1.6)	0 (0)	0 (0)	
Negative AMA-M2 (N, %)	NT	95 (100)	63 (100)	15 (100)	17 (100)	NA
Ceruloplasmin (g/L)	NT	0.30 (0.24, 0.35)	0.29 (0.24, 0.34)	0.29 (0.23, 0.42)	0.34 (0.24, 0.53)	.27
Ferritin (ng/mL)	NT	205.95 (128.33, 369.00)	212.90 (104.90, 373.40)	322.20 (162.00, 530.80)	133.45 (98.53, 185.10)	.022
TS (%)	NT	46.93 ± 19.28	48.65 ± 21.36	48.35 ± 14.07	36.56 ± 10.23	.16
AFP (ng/mL)	NT	3.90 (2.90, 6.24)	4.17 (2.98, 8.14)	3.56 (2.40, 4.44)	3.41 (2.60, 5.13)	.109
WBC (×10 ⁹ /L)	7.1±1.9	5.6±2.1	5.7±2.2	5.7±1.2	5.5±2.5	.98
Platelets (×10 ⁹ /L)	194.4±59.6	203.2±58.3	210.6±56.5	192.8±55.9	184.8±67.7	.65
Hemoglobin (g/L)	128.8±8.4	132.4±17.0	135.0±16.8	133.4±13.4	123.2±18.4	.34
RUCAM score	NT	6 (5, 7)	7 (6, 8)	5 (4, 6)	4 (4, 6)	< .001
Severity score	NT	2 (1, 3)	2 (1, 3)	3 (1, 3)	2 (1, 3)	.40

Footnote: [#]**P value** stands for the overall comparison of hepatocellular, mixed and cholestatic phenotype of DILI; **AFP**, alpha fetoprotein;

ALP, alkaline phosphatase; **ALT**, alanine aminotransferase; **AMA-M2**, anti-mitochondrial M2 antibody; **ANA**, anti-nuclear antibody;

AST, aspartate aminotransferase; **BMI**, body mass index; **DBil**, conjugated bilirubin; **GGT**, gamma-glutamyl transferase; **Ig**,

immunoglobulin; **INR**, international normalized ratio; **NA**, not available for statistically significance analysis; **NT**, not tested; **RUCAM**,

Roussel Uclaf Causality Assessment Method; **TBA**, total bile acids; **TBil**, total bilirubin; **TS**, transferrin saturation; **WBC**, white blood

cells.

Table 2 Serum TCA level was an independent risk factor for days to resolution by Cox-regression analysis

Significant factors in univariate-analysis	Univariate		Multivariate	
	HR (95% CI)	P value	HR (95% CI)	P value
Severity	0.75 (0.62, 0.91)	.004	0.90 (0.69, 1.16)	.41
TBA (>31.85µmol/L vs. <31.85µmol/L)	0.65 (0.43, 0.98)	.041	0.83 (0.41, 1.67)	.59
GCA (>5463.00nmol/L vs. <5463.00nmol/L)	0.58 (0.38, 0.88)	.010	0.49 (0.16, 1.5)	.21
TUDCA (>1073.60nmol/L vs. <1073.60nmol/L)	0.56 (0.37, 0.85)	.007	1.06 (0.46, 2.42)	.90
THCA (>109.65nmol/L vs. <109.65nmol/L)	0.59 (0.39, 0.89)	.013	0.89 (0.41, 1.93)	.76
TCA (>1955.41nmol/L vs. <1955.41nmol/L)	0.47 (0.31, 0.73)	.001	3.04 (1.08, 8.58)	.036
TLCA-3S (>360.40nmol/L vs. <360.40 nmol/L)	0.56 (0.37, 0.87)	.009	1.11 (0.65, 1.89)	.72
NorCA (>22.29nmol/L vs. <22.29nmol/L)	0.58 (0.38, 0.87)	.009	1.40 (0.81, 2.41)	.22
β CDCA (>23.03nmol/L vs. <23.03nmol/L)	1.82 (1.19, 2.78)	.006	0.75 (0.39, 1.44)	.39

Footnote: βCDCA, 3β-chenodeoxycholic acid; CI, Confidence Interval; GCA, glycocholic acid; HR, Hazard Ratio; NorCA, nor cholic

acid; TBA, total bile acid; TCA, taurocholic acid; THCA, taurohyocholate; TLCA-3S, tauroolithocholic acid 3 sulfate; TUDCA,

tauroursodeoxycholic acid.

Table 3 Addition of serum TCA level could improve predictive ability of 6-month abnormal biochemistry in DILI patients

Evaluating indexes	AUROC (95% CI)	Optimal cut-off value	Sensitivity (95% CI), %	Specificity (95% CI), %	Positive likelihood ratio	Negative likelihood ratio	<i>P</i> value when compared with [#]
Age (years)	0.58 (0.47-0.68)	53	62.50 (35.40 - 84.80)	53.16 (41.60 - 64.50)	1.33	0.71	0.0044
Severity	0.65 (0.54-0.74)	2	68.75 (41.30 - 89.00)	58.23 (46.60 - 69.20)	1.65	0.54	0.11
TCA (nmol/L)	0.69 (0.59-0.78)	1955.41	81.25 (54.40 - 96.00)	56.96 (45.30 - 68.10)	1.89	0.33	0.0009
Severity-age [#]	0.75 (0.65-0.83)	0.13	75.00 (47.60 - 92.70)	59.49 (47.90 - 70.40)	1.85	0.42	0.37
TCA-severity-age	0.81 (0.71-0.88)	0.17	68.75 (41.30 - 89.00)	81.01 (70.60 - 89.00)	3.62	0.39	/

Footnote: AUROC, areas under the receiver operating characteristic curves; CI, confidence interval; TCA, taurocholic acid.